Microwave-assisted extraction and hydrolysis: An alternative tool to pyrolysis for the analysis of recalcitrant organic matter? Application to a forest soil (Landes de Gascogne, France)

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Abstract

A comparison was made between the composition of the recalcitrant organic matter (ROM) isolated from a sandy forest soil as revealed by microwave assisted extractions and/or hydrolyses and by usual pyrolysis techniques. Successive microwave irradiation treatments were performed in H_2O, 0.1 and 1 M HCl and 0.1 and 1 M KOH. At each step of the treatment the insoluble residue was examined via Curie point pyrolysis (CuPy) and Curie point thermally assisted hydrolysis and methylation (CuTHM). Sequential irradiation treatments resulted in ca 35% degradation of the ROM. Compounds released on microwave irradiation in H_2O and in HCl were dominated by glucose, suggesting the occurrence of carbohydrate-containing molecular associations in the soil organic matter (SOM) which were not disrupted during acid hydrolyses and extractions as applied for the isolation of the ROM. The product distribution from the microwave irradiation in KOH showed an important contribution to the ROM from the higher plant polyesters cutin and suberin, and to a lesser extent from lignin. Different lignin-derived compounds were specifically released upon microwave acid or base hydrolyses. This suggested that two types of lignin monomers, ether or ester linked, occurred in the ROM. The changes observed in the composition of the CuPy pyrolysates of the residues from the different microwave hydrolyses are consistent with the near complete removal of carbohydrates by microwave HCl hydrolysis. The changes observed in the composition of the CuTHM pyrolysates of the residues from the different microwave acid and base hydrolyses are in agreement with a major release of cutin- and suberin-derived compounds upon microwave KOH hydrolysis. The CuPy and CuTHM pyrolysates of the final residue consists predominantly in lignin-derived compounds. This study emphasizes the potential of microwave-assisted hydrolyses to give a better estimate of the actual contribution

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of cutin to the ROM than pyrolysis. However, this technique appears to be unable to completely release the lignin-based constituent of the ROM. Microwave irradiation appears to provide great potential as a tool for extraction and chemical characterization of complex OM and could be an attractive additional technique to pyrolyses.

1. Introduction

Soil organic matter (SOM) is one of the major carbon pools playing an important role in the global carbon cycle. It contains a chemically recalcitrant (i.e. inert towards drastic laboratory acid hydrolyses) organic matter fraction (ROM). This recalcitrant, non-hydrolysable SOM fraction may account for a substantial part of the total organic carbon of the soil (Poirier et al., 2002, 2003; Quénéa et al., 2005a, 2006a; Mikutta et al,. 2006) and can contribute to the stable carbon pool in the soil (Paul et al., 1997, 2006; Mikutta et al., 2006). The inherent or acquired recalcitrance of the ROM fraction and consequent long mean residence time in the soil might contribute to the potential role of SOM as a sink for atmospheric CO_2. The chemical composition and origin of ROM have been chiefly studied using spectroscopic and pyrolytic methods (Poirier et al., 2002, 2003; Naafs, 2004; Quénéa et al., 2005a, 2006a, b). Few chemical degradation studies have been applied to characterise its composition (Quénéa et al., 2005b; Naafs, 2004; Winkler et al., 2005). These studies have pointed out the diversity of aliphatic structures making up the ROM network and the important role of ester functions to link these constituents. Aliphatic constituents are usually considered to derive predominantly from the biopolyesters cutin and/or suberin (Quénéa et al., 2005a,b) or from cutan and/or suberan (Augris et al., 1998; Naafs, 2004), the recalcitrant biopolymers originating from plant cuticles and suberized plant cell walls respectively (Nip et al., 1986; Tegelaar et al., 1995). Some of the pyrolytic studies have also pointed out the significant preservation of polysaccharide-type materials in the ROM (Quénéa et al., 2005a, 2006b). However, one of the main drawbacks of the usually on-line pyrolytic techniques performed is the lack of quantitative data about abundances of compounds released upon pyrolysis.

Microwave assisted extraction of organic compounds from matrices such as soils, seeds or food was introduced by Ganzler et al. (1986). This extraction technique has been extended to environmental analysis of contaminants in soils, sediments or water and to the extraction of natural products (e.g. Letellier and Budzinski, 1999; Camel, 2000). Microwaves are high frequency electromagnetic waves which are strongly absorbed by polar molecules. Absorption results in rapid and intensive dielectric heating. Microwave systems using closed

vessels can operate at elevated temperature and pressure and temperature of solvents submitted to microwave irradiation can be raised above their boiling point (e.g. Letellier and Budzinski, 1999). The complex macromolecular recalcitrant fraction of SOM contains polar constituents and strong localised heating can be expected to occur at these polar targets under microwave irradiation. This could result in extraction and/or release of some of the constituents of the matrix, opening up a new analytical possibility for obtaining information on the chemical composition of such macromolecular material.

In this paper a comparison was made between microwave assisted extraction and/or hydrolysis and pyrolytic methods for the chemical characterisation of recalcitrant organic matter. Capabilities of the two methods were illustrated by analysis of the ROM isolated from a sandy forest soil. The compositions of products released after neutral, acid and base microwave irradiation treatments were analysed. We compared these compositions to those of products released upon standard Curie point pyrolysis (CuPy/GC-MS) and Curie point thermally assisted hydrolysis and methylation (CuTHM) with tetramethylammonium hydroxyde (TMAH). The comparison was used to evaluate the potential of the two methods for chemical characterization of complex organic matter.

2. Materials and methods

All chemicals used were analytical grade.

2.1. Sample

The ROM sample was isolated from a soil collected from a maritime pine (*Pinus pinaste*r) forest. The dominant forest undergrowth was composed of ferns (*Pteridium aquilinum)* and perennial grasses (*Molinia coerulea*; Jolivet, 2000). The isolation protocol and bulk features of the ROM have been previously described (Quénéa et al., 2005a). Briefly, lipid- and humic substance-free soil was submitted to stepwise acid hydrolyses using trifluoroacetic acid and hydrochloric acid. The hydrolysed material was demineralised using HCl/HF treatment. The ROM was recovered as the insoluble residue remaining after neutralisation and extraction with $CHCl_3$/MeOH (2/1, v/v). The ROM accounted for 1.6% of the whole soil, i.e. about 34% of the total initial carbon. (Quénéa et al., 2005a). Its elemental composition was 58.7% C; 4.2% H; 1.0% N; 4.5% ash (Quénéa et al., 2005a).

2.2. Microwave assisted extraction and hydrolysis

An outline of the sequential microwave assisted extractions and hydrolyses is shown in Fig. 1. The treatments were performed with a single-mode CEM Discover ® microwave reactor at a frequency of 2450 MHz (0-300 W) in closed reaction vessels. The temperature was measured with an infrared sensor outside the reaction vessel. The samples were subjected to 20 irradiation cycles. An irradiation cycle consisted in an irradiation period of 40 s followed by a phase of cooling, without transfer of microwave energy, for 1 min.

The ROM (ca. 100 mg) in 2 ml H_2O was subjected to the irradiation cycles using 100 W microwave energy. The maximum temperature and pressure were 140 °C and 2.5 10^5 Pa respectively. After the irradiation cycles, the reaction vessel was cooled to room temperature with compressed air and the reaction mixture extracted at room temperature during 4 h with CH_2Cl_2/MeOH (1:1 v/v; 30 ml). The reaction mixture was centrifuged at 1,400 g for 15 min. The extract **1** was dried under reduced pressure, treated with 4 M HCl in MeOH [prepared by mixing CH_3COCl with MeOH (1:2.5 v/v)] for 1 h at 60 °C to esterify carboxyl groups and then with a mixture of pyridine/*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 10:1 v/v) for 10 min at 60 °C to convert hydroxyl groups to trimethylsilyl ethers.

The insoluble residue **1** from the CH_2Cl_2/MeOH extraction was dried, transferred to a reaction vessel and 2 ml 0.1 M HCl were added. The reaction mixture was subjected to 20 irradiation cycles using 100 W microwave energy. The maximum temperature and pressure were 150 °C and 10^6 Pa respectively. After irradiation, H_2O (10 ml) was added and the reaction mixture was centrifuged at 1,400 g for 15 min. An aliquot of the aqueous extract was dried under reduced pressure and derivatized with BSTFA before GC-MS analysis. Another aliquot was reduced with $NaBH_4$ (50 mg) for 1 h at room temperature (Albersheim et al., 1967). The resulting products (alditols) were silylated with BSTFA. These alditols were identified using GC comparison with trimethylsilyl ethers of standard alditols. The pellet was extracted at room temperature during 4 h with CH_2Cl_2/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g for 15 min. The organic phase (extract **2**) was dried under reduced pressure and the products derivatised with HCl/MeOH and BSTFA. Aliquots of the dried residue **2** (ca. 3 mg) were analysed using CuPy/GC-MS and CuTHM/GC-MS.

The remaining residue **2** was subjected to 20 irradiation cycles with 100 W microwave energy in 2 ml 1 M HCl. The maximum temperature and pressure were 150 °C and 9 10^5 Pa respectively. After irradiation, H_2O (10 ml) was added and the reaction mixture was centrifuged (1,400 g, 15 min). The aqueous extract was dried under reduced pressure, derivatised with BSTFA and analysed using GC-MS. The pellet was extracted at room

temperature during 4 h with CH_2Cl_2/MeOH (1:1 v/v; 30 ml) and centrifuged (1,400 g, 15 min). The extract **3** was dried under reduced pressure and the products derivatised with HCl/MeOH and BSTFA. Aliquots of the residue **3** were analysed using CuPy/GC-MS and CuTHM/GC-MS. The residue **3** was subjected to 20 irradiation cycles with 100 W microwave energy in 2 ml 0.1 M KOH. The maximum temperature and pressure were 150 °C and 6 10^5 Pa respectively. After irradiation, the reaction mixture was acidified with 1 M HCl and centrifuged at 1400 g for 15 min. The pellet was extracted at room temperature during 4 h with CH_2Cl_2/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g for 15 min. The supernatant (extract **4**) was dried under reduced pressure and the products derivatised with HCl/MeOH and BSTFA. The residue **4** was subjected to 20 irradiation cycles with 100 W microwave energy in 1 M KOH. The maximum temperature and pressure were 150 °C and 5 10^5 Pa respectively. The reaction mixture was acidified with 1 M HCl and centrifuged at 1,400 g for 15 min. The pellet was extracted at room temperature during 4 h with CH_2Cl_2/MeOH (1:1 v/v; 30 ml) and centrifuged (1,400 g, 15 min). The supernatant (extract **5**) was dried under reduced pressure and the products derivatised with HCl/MeOH and BSTFA. The residue **5** was analysed using CuPy/GC-MS and CuTHM/GC-MS. Yields were determined by weighting the extracts and residues.

2.3. Analytical techniques

Gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer with electron ionization at 70 eV. Separation was achieved using a fused silica column coated with DB-5MS (30 m, i.d. 0.25 mm, film thickness 0.5 μm) with He as carrier gas. The GC oven was programmed from 100 °C to 320 °C at 4 °C min^{-1}. Compound identification was based on the NIST mass spectrum library or interpretation of the spectra and comparison of GC retention times with those of standards. When determined, the relative abundances of the positional isomers of mid-chain hydroxyl alkanoic acids and mid-chain alkanedioic acids (compounds **23**, **24**, **26**, **27** and **29** in Table 1) were estimated using the mass spectral α-fragmentation at the secondary hydroxy group yielding the most intense fragment ion *m/z* $[MeOOC(CH_2)_nCHOTMSi]^+$ and assuming that the sensitivity was the same for each isomer.

2.4. Pyrolytic studies

Curie point pyrolysis gas chromatography-mass spectrometry (CuPy/GC-MS) was performed using a Pilodist Curie point pyrolyzer. Samples (ca. 1 mg) were pyrolysed for 10 s using ferromagnetic wires with a Curie temperature of 610 °C under a He flow of 5 ml min^{-1}. The pyrolysis unit was directly coupled to the GC-MS system. The pyrolysis products were separated using a Thermo Trace GC Ultra gas chromatograph equipped with a 30 m Rtx-5Sil MS column (0.25 mm i.d., 0.5 µm film thickness). The oven temperature was held at 50 °C for 10 min and raised to 300 °C at 2°C min^{-1}. The gas chromatograph was coupled to a thermo DSQ mass spectrometer operating at 70 eV. For Curie point thermally assisted hydrolysis and methylation (CuTHM) with tetramethylammonium hydroxide (TMAH), the same GC-MS conditions as above were used. Samples (ca. 1 mg) were mixed with 100µL TMAH (25% w/w in H_2O), partially dried under reduced pressure and loaded on the ferromagnetic wire (Curie temperature 610 °C).

3. Results

3.1.Microwave irradiation conditions

The microwave reactor operated up to 2×10^6 Pa. In the case of higher pressures the system was automatically vented. In the closed vessels used in this study, the maximum allowed pressure corresponded to maximum temperatures of ca. 220 °C and 200 °C in H_2O and 0.1 M HCl respectively. Preliminary experiments on humic acids (unpublished results) showed that, upon continuous microwave irradiation in H_2O at 220 °C for 30 min, the temperature (corresponding to a pressure of ca. 1.7 10^6 Pa) was rapidly reached (< 1 min). When the temperature was reached the microwave energy strongly decreased (ca. 5-20 W). A two fold increase in the irradiation time did not improve the yield of extraction. Conversely, the use of several periods of irradiation followed by a cooling phase allowed to keep a high microwave energy during a longer time and increased extraction yield (ca. 10% increase in the case of the studied humic acids). Although we were aware that optimum irradiation energy, temperature and numbers of cycles depended on the sample matrix, optimisation study was not carried out for the ROM sample studied owing to the small available amounts of this sample. In this study we used cycles consisting of irradiation at 100 W for 40 s followed by a cooling phase of 1 min.

3.2. Microwave irradiation in H_2O

The microwave assisted H_2O extraction yielded very low amounts of products (1-2% of the initial ROM). These products (Fig. 2a and Table 1 for peak annotations) were largely dominated by monosaccharides. Small amounts of disaccharides were also detected at longer retention times. Apart from predominant sugars, the extract afforded two sets of compounds. The first one contained aliphatic compounds. It included (i) *n*-alkanoic acids ranging from C_{14} to C_{26} with C_{16} and C_{18} as the major components. A C_{18} monounsaturated acid was also present in the extract. (ii) *n*-alkan-1-ols ranging from C_{16} to C_{26} and dominated by C_{18} and C_{24}, (iii) ω-hydroxy acids ranging from C_{16} to C_{26} with C_{16} and C_{22} as major components and mid-chain hydroxy acids consisting of 8/9/10-hydroxy octadecanoic acid (**23**), 8/9/10,16-dihydroxy hexadecanoic acid (**27**) and 9,10-dihydroxy octadecanoic acid (**28**). 8-, 9- and 10-Hydroxy octadecanoic acids accounted respectively for ca. 10, 45 and 45% of the total isomeric mixture **23** (see *2.4* of Materials and Methods). 10,16- Dihydroxy hexadecanoic acid was the major isomer (ca. 60% of the total isomeric mixture **27**) followed by 9,16- and 8,16-dihydroxy hexadecanoic acids (ca. 30 and 10% of the isomeric mixture respectively). The second set contained aromatic compounds consisting of substituted phenols (**4**, **9**, **12**; Table 1), substituted 2-methoxyphenols (**7**, **14**, **15**, **16**) and substituted 2,6-dimethoxyphenol (**13**). Based on their mass spectra (Fig. 3), compounds **14** and **15** were considered to belong to the the guaicyl units. The relative abundances of *p*-hydroxyphenyl, guaiacyl and syringyl units were estimated using the peak areas in the mass chromatogram, assuming a similar sensitivity for each compound. Guaiacyl units dominated the aromatics (ca. 45% of the total aromatics including ca. 4% ferulic acid) followed by *p*-hydroxyphenyl (ca. 20% of the total aromatics including 11% *p*-coumaric acid) and syringyl units (ca. 11% of the total aromatics).

3.3. Microwave irradiation in HCl

We could not undoubtedly state that products released using microwave irradiation in aqueous HCl originated from hydrolysis rather than from an extraction process. However, comparison of the product distributions between microwave irradiation in HCl and in H_2O (see Discussion) suggested that a proportion of the products released from microwave irradiation in HCl likely originated from acid hydrolysis of some constituents of the ROM. Therefore, in this paper, we termed microwave irradiation in HCl as microwave HCl hydrolysis, keeping in mind that this term likely grouped hydrolysis and extraction together.

3.3.1. Microwave irradiation in 0.1 M HCl

The products released upon microwave irradiation in HCl 0.1 M accounted for ca. 7% of the residue **1** remaining after microwave irradiation in H_2O and consisted mainly of monosaccharides. The aqueous extract (Fig. 1) accounted for ca. 70% of the products released upon microwave irradiation in 0.1 M HCl and consisted predominantly of monosaccharides. Low abundances of disaccharides and trace amounts of amino acids (mainly aspartic acid and 5-oxoproline) were also detected through GC-MS. Analysis of the trimethylsilyl ethers of the alditols of the aqueous extract revealed that glucose was by far the predominant monosaccharide present in the aqueous extract from microwave 0.1 M HCl hydrolysis. Xylose and mannose were observed at extremely low levels. Arabinose and galactose were not found.

The organic extract (extract **2**) was also largely dominated by monosaccharides (Fig 2b), glucose being the major one based on retention times. Apart from the largely dominant glucose, the organic extract from microwave 0.1 M HCl hydrolysis gave two additional compound classes (Fig. 2b and Table 1 for peak annotations). The first one consisted of aliphatic compounds and included (i) *n*-alkanoic acids ranging from C_{14} to C_{32} with C_{16} and C_{18} as major components. A C_{18} monounsaturated acid was also detected,(ii) *n*-alkan-1-ols from C_{12} to C_{32} with C_{22} and C_{24} as major components, (iii) ω-hydroxy alkanoic acids ranging from C_{16} to C_{28} and dominated by C_{22}, (iv) mid-chain hydroxy alkanoic acids comprising 8/9/10-hydroxy octadecanoic acid (each isomer accounted respectively for ca. 10, 45 and 45% of the isomeric mixture **23**), 8/9/10,16-dihydroxy hexadecanoic acid (each isomer accounted respectively for ca. 10, 30 and 60% of the isomeric mixture **27**) and 7/8-hydroxy hexadecanedioic (**26**). The relative abundances of these isomers were respectively ca. 40 and 60% of the isomeric mixture **26**. α,ω-Alkanedioic acids were also detected in trace amounts. The second class contained aromatic compounds comprising substituted phenols (**1**, **2**, **3**, **4**, **9**, **12**), substituted 2-methoxyphenols (**6**, **7**, **16**) and substituted 2,6-dimethoxy phenols (**13**). On the basis of their mass spectra tentatively identified and unidentified compounds (**8**, **14**, **15**, **17**, **18** and **19**, Fig. 3) were considered to belong to this aromatic class. This class was largely dominated by guaiacyl units (ca. 57% of the total aromatics including 1% ferulic acid). *p*-Hydroxy phenols (including 2% *p*-coumaric acid) and syringyl units accounted for ca. 8 and 16% of the total aromatics, respectively. Isomeric cyclodimers of *p*-coumaric acid (**20**) were also detected in small amounts. The mass spectra of isomers **20** (as methyl ester, trimethylsilyl ether derivatives) were similar and exhibited the same major ions (*m/z* 250,

235) as in the mass spectrum of the methyl ester, trimethysilyl ether of *p*-coumaric acid and their fragmentation patterns (Fig.3g) were consistent with cyclodimers of *p*-coumaric acid (Ford and Hartley, 1989).

3.3.2. Microwave irradiation in 1 M HCl

The products released upon microwave irradiation in 1 M HCl accounted for ca. 6% of the residue **2** remaining after microwave irradiation in 0.1 M HCl. The aqueous extract (ca. 60% of the products released upon microwave irradiation) and the organic extract (extract **3**) were qualitatively the same as those resulting from microwave irradiation in 0.1 M HCl. Glucose largely dominated both aqueous and organic extracts. In the aqueous extract, disaccharides were hardly detected and trace amounts of the same amino acids as in the aqueous extract from microwave irradiation in 0.1 M HCl were present (data not shown). Similar aromatic and aliphatic compounds were identified in the organic extract **3** (data not shown). However, the relative abundances of the aromatic and aliphatic compounds were much lower than in the organic extract from the microwave irradiation in 0.1 M HCl.

3.4. Microwave irradiation in KOH

As in the case of microwave irradiation in HCl, it could be assumed that hydrolysis (particularly of esters) occurred upon microwave irradiation in KOH. Microwave irradiation of the residue **3** in 0.1 M KOH yielded very low amounts of products. The organic extract (extract **4**) accounted for ca. 1% of the residue. By contrast, the organic extract from microwave irradiation in 1 M KOH (extract **5**) accounted for 22% of the residue **4**. Similar products were identified in both extracts, so only the products released upon microwave irradiation in 1 M KOH were presented here. Fig. 2c depicted the distribution of the products extracted from the microwave 1 M KOH hydrolysate (extract **5**). The microwave KOH hydrolysis yielded two main compound classes. The first contained aliphatic compounds which largely dominated the extract. It consisted in (i) *n*-alkanoic acids ranging from C_{14} to C_{32} with C_{16}, C_{18} and C_{28} as the major constituents. A C_{18} monounsaturated acid was also present, (ii) *n*-alkan-1-ols from C_{16} to C_{32} with maximum at C_{22} and C_{24}, (iii) ω-hydroxy alkanoic acids ranging from C_{14} to C_{28} and maximising at C_{22}. A monounsaturated C_{18} ω-hydroxy acid was also detected in low amounts, (iv) mid-chain hydroxy alkanoic acids (**21**, **22**, **23**, **25**, **27**, **28**, **29**, **30**, Table 1 for peak annotations) dominated by 8/9/10,16 dihydroxy

hexadecanoic acids (each isomer accounted respectively for ca 10, 30 and 60% of the isomeric mixture **27**) and hydroxy alkanedioic acids (**24**, **26**) comprising C_{15} and C_{16} homologues with 7/8-hydroxy hexadecanedioic (each isomer accounted for ca. 40 and 60% of the isomeric mixture **26**) as the major component. α,ω-Alkanedioic acids ranging from C_{16} to C_{28} with C_{22} and C_{24} as the major constituents were also detected in trace amounts. The second class comprised aromatic compounds consisting of substituted phenols (**1**, **2**, **3**, **4**, **9**, **10**, **12**, Table 1 for peak annotations), substituted 2-methoxyphenols (**5**, **6**, **7**, **14**, **16**) and substituted 2,6-dimethoxyphenols (**13**). Isomeric cyclodimers of *p*-coumaric acid (**20**) were also present in substantial amount. This compound class was dominated by guaiacyl units (ca. 46% of the total aromatics, including 9% ferulic acid) and *p*-hydroxy phenols (ca. 38% of the total aromatics, including 29% *p*-coumaric acid). Syringic units accounted for ca. 6% of the total aromatics.

3.5. Pyrolytic study of the residues

In order to substantiate the efficiency and specificity of the microwave irradiation under acidic and basic conditions, the insoluble residues obtained after 0.1, 1 M HCl and 1 M KOH microwave hydrolyses (residues **2**, **3** and **5**) were subjected to Curie point pyrolysis (CuPy/GC-MS) and thermally assisted hydrolysis and methylation (CuTHM/GC-MS). Microwave irradiation in H_2O and in 0.1 M KOH releasing very low amounts of products, therefore, the CuPy/GC-MS and CuTHM/GC-MS of the corresponding residues were not performed.

3.5.1. CuPy/GC-MS

CuPy/GC-MS traces for the ROM and for the residues **2**, **3** and **5** were shown in Fig. 4. The identified products were listed in Table 2. The ROM pyrolysate (Fig. 4a) was characterised by the presence of four main compound classes. The first was constituted of furans (**1**, **2b**, **8**), levoglucosenone (**6**) and levoglucosan (**15**) which were generally considered as polysaccharide pyrolysis products. Aromatic compounds (**2a**, **3**, **4**, **5**, **7**, **9**-**14**, **16**), likely related to lignin, constituted the second class. The third class was composed of *n*-alkane/*n*-alkene doublets. The series, ranging from C_9 to C_{32} did not show any clear carbon number predominance, except the relatively high abundance of the C_{20}, C_{22} and C_{24} homologues. The

last class was constituted by *n*-alkan-2-one/*n*-alken-2-one doublets. The series, with a strong odd/even predominance, ranged from C_{19} to C_{35} with C_{29} and C_{27} as the major components.

The pyrochromatogram of the residue obtained after microwave 0.1 M HCl hydrolysis (Fig. 4b; Table 2 for peak annotations) shown a strong decrease in the relative abundance of levoglucosan (**15**). The decrease in the relative abundances of the other compounds related to polysaccharides (i.e. **1**, **2b**, **6**, **8)** was more pronounced after microwave 1 M HCl hydrolysis (Fig. 4c; Table 2 for peak annotations). Within the other classes (aromatic, *n*-alkane/*n*-alkene and *n*-alkan-2-one/*n*-alken-2-one), the relative abundances of the constituents appeared to remain rather constant after these irradiations.

The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolysis (Fig. 4d) shown a strong decrease of the relative abundances of the *n*-alkane/*n*-alkene and *n*-alkan-2-one/*n*-alken-2-one doublets. It was dominated by 2-methoxy phenol (**5**) and 4-methyl-2-methoxyphenol (**7**).

3.5.2. CuTHM/GC-MS

Fig. 5 shown the total ion current (TIC) chromatograms of the thermochemolysates of the ROM and of the residues **2**, **3** and **5**. The compounds identified as methyl esters and methyl ethers were listed in table 3.

The CuTHM pyrolysate of the ROM (Fig. 5a) was quite similar to that obtained by Quénéa et al. (2005a). Two main product classes constituted this pyrolysate. The first was constituted by aromatic compounds (**4**-**19**, **27**) which were dominated by the methylated counterparts of vanillic acid (**14**) and *p*-coumaric acid (**17**). Isomeric cyclodimers of *p*-coumaric acid (**27**) were also present at longer retention time. The second class contained aliphatic compounds including as the major constituents: (i) *n*-alkanoic acids (a C_{18} monounsaturated acid was identified), ranging from C_{16} to C_{32} with a strong even carbon number predominance. The distribution was dominated by long chain components ($>C_{20}$); (ii) *n*-alkan-1-ols ranging from C_{20} to C_{32} with a strong even/odd predominance and a maximum at C_{22}. (iii) ω-hydroxy acids ranging from C_{16} to C_{30} with an even/odd predominance and C_{22} and C_{24} as the major components; (iv) long chain α,ω-alkanedioic acids, present in lower relative abundances, ranging from C_{20} to C_{28}. The distribution exhibited an even/odd predominance and was dominated by C_{24}; (v) mid-chain hydroxy alkanoic and hydroxy alkanedioic acids (**20**-**26,** Table 3 for peak annotation). The tentative identification of these compounds was based on the interpretation of their mass spectra (Fig. 6). When several structures were possible, the

carbon chain lengths found for the mid-chain hydroxy compounds identified in the extracts were selected (i.e. C_{15}, C_{16} and C_{18}). The resulting identification suggested an incomplete methylation of some hydroxyl groups (compounds **22**, **24** and **26**). A minor series of *n*-alkane/*n*-alkene doublets ranging from C_{11} to C_{33} with no obvious carbon number predominance was also identified.

The pyrochromatograms of the residues obtained after microwave HCl hydrolyses shown a decrease in the relative abundances of the aliphatic compounds (Fig. 4b, c) with the exception of *n*-alkane/*n*-alkene doublets whose relative abundances appeared to increase. The decrease in the relative abundances of aliphatic compounds was clearly more pronounced after microwave KOH hydrolysis (Fig. 4d).

The TIC trace for the residue remaining after microwave irradiation in 1 M KOH was largely dominated by aromatic compounds with vanillic acid (**14**) and *p*-coumaric acid (**17**) as the major constituents. Within the aliphatic compound class, the decrease in the relative abundances of ω-hydroxy acids resulting from microwave KOH hydrolysis, appeared to be stronger than that of the other constituents (e.g. alkanoic acids or alkanols).

4. Discussion

4.1. Comparison between microwave irradiation in H_2O and HCl. Extraction or hydrolysis?

Although we cannot completely rule out the possibility of bond cleavage under the influence of temperature and pressure, we can assume that products released on microwave irradiation in H_2O arose mainly from a desorption process. It is then likely that monosaccharides, lignin-derived and aliphatic compounds found in the H_2O extract are present as such in the ROM. These compounds, likely adsorbed on (or entrapped in) the macromolecular structure of the ROM, were not released during the isolation of the ROM despite the drastic acid hydrolyses applied (Quénéa et al. 2005a). This could indicate that microwave energy interacts efficiently with either the macromolecular structure or the adsorbed (or entrapped) compounds and is able to disrupt such interactions. Contrary to the extract from microwave irradiation in H_2O and 0.1 M HCl, disaccharides were hardly detected in the extract from microwave irradiation in 1 M HCl, suggesting that at least part of the monosaccharides in 1 M HCl extract arises from an acid hydrolysis of oligo- and/or polysaccharides. The occurrence of acid hydrolysis during microwave irradiation in HCl is also supported by the presence of minute amounts of amino acids which were not observed in the extract from microwave irradiation in H_2O. The

predominance of monosaccharides in the extracts from microwave irradiation in H_2O and HCl indicates that intact monosaccharides, and probably oligo- and polysaccharides, are still present in the ROM despite the intensive trifluoroacetic acid and HCl hydrolyses performed during isolation (Quénéa et al., 2005a).

The GC-MS traces revealed a very similar distribution of aliphatic compounds in the extracts from microwave irradiation in both 0.1 M HCl and H_2O (Figs. 2a, b). By contrast, some differences are observed for the aromatic compound distributions between these extracts. The greatest difference is a greater range of aromatic compounds in the extract from microwave HCl hydrolysis. Seventeen aromatic compounds and cyclodimers of *p*-coumaric acid were identified in the extract from microwave irradiation in 0.1 M HCl while only eight aromatic compounds were present in the extract from microwave irradiation in H_2O. This difference, together with the strong decrease in the relative abundances of disaccharides and the presence of amino acids in the extracts from microwave HCl hydrolyses, suggest that hydrolysis of some constituents of the ROM, in this case, carbohydrate, protein and lignin moieties, occurred upon microwave irradiation in HCl.

4.2. Origin of aliphatic compounds

Aliphatic compounds in all the extracts from microwave irradiation in H_2O, HCl and KOH, are well known constituents of cutin and suberin. The polyester cutin, present in the majority of the aerial parts of vascular plants, and suberin, present in the bark and roots of vascular plants, differ in the chain length and the substitution patterns of their monomers (Walton, 1990; Kolattukudy, 2001). Long chain ($\geq C_{20}$) *n*-alkanedioic acids, ω-hydroxy acids, *n*-alkanoic acids and, to a lesser extent, *n*-alkan-1-ols are frequently dominant monomers of suberin, while mid-chain substituted monomers are usually minor constituents. On the contrary, cutin is characterised by substantial abundances of C_{16} and C_{18} mid-chain substituted monomers, while long chain monomers ($\geq C_{20}$) are rarely present. In the extracts from microwave irradiation in H_2O and in HCl (Figs. 2a, b), the distribution patterns of aliphatic compounds are quite similar. Apart from ubiquitous C_{16} and C_{18} *n*-alkanoic acids, the aliphatic compounds are dominated by long chain ($\geq C_{20}$) components which can be considered as predominantly from a suberin source. However, the high relative abundances of C_{22} and C_{24} *n*-alkan-1-ols suggests they could also originate from the plant wax esters in

which they are widespread (Bianchi, 1995). The mid-chain hydroxy constituents, probably originating from cutin, are present in lower amounts.

Microwave irradiation in KOH is more efficient for promoting hydrolysis of ester linkages of cutin and suberin than microwave irradiation in HCl. Therefore, the distribution pattern of aliphatic compounds in the extract from 1 M KOH hydrolysis (Fig. 2c) strongly differs from that of aliphatic compounds in the extracts from microwave irradiation in HCl (Fig. 2b). ω-Hydroxy alkanoic acids with chain length ≥ C_{20}, likely originating from suberin, largely dominate. In addition, this extract contains C_{15}, C_{16} and C_{18} mid-chain hydroxy alkanoic and C_{15} and C_{16} mid-chain alkanedioic acids in high amounts. This indicates a significant contribution of cutin to the ROM. The contribution of cutin from pine, the predominant vegetation, is indicated by the presence of trace amounts, of C_{12} and C_{14} ω-hydroxy alkanoic acids, which have been reported as typical constituents of cutin of the needles of numerous species of gymnosperms (Matzke and Riederer, 1991; Nierop, 2001; Nierop and Verstraten, 2004; Otto and Simpson, 2006). The contribution of cutin from pine needles could be also suggested by the presence of mid-chain hydroxy pentadecanoic acids (**21**, **22**, **25;** Table 1) and pentadecanedioic acid (**24;** Table 1), which have been reported as monomers of cutin of some gymnosperms (Hunneman and Eglinton, 1972), pine needles and grasses (Otto and Simpson, 2006). The dominant contribution of 8,16- and 10,16-dihydroxy hexadecanoic acid to the dihydroxy hexadecanoic isomeric mixture (respectively ca. 30 and 60% of the isomeric mixture **27** in all the extracts) suggests a significant contribution of cutin from the undergrowth (predominantly grasses and ferns). Indeed according to Goñi and Hedges (1990), in contrast to most of the gymnosperm species, Poaceae species produced high yield of cutin-derived 8,16- and 10,16-dihydroxy hexadecanoic acids. Moreover, 10,16-dihydroxy hexadecanoic acid has been reported as the most abundant component in the solvent-extracted soil under grasses and ferns (Naafs and van Bergen, 2002) The relative contributions of suberin and cutin to the ROM may be estimated using monomers typical of cutin or suberin and monomers common to both polyesters as suggested by Otto and Simpson (2006). The suberin/ cutin ratio of 1.8 calculated in this way from the microwave 1 M KOH hydrolysis for the ROM corroborates the predominance of suberin input previously reported (Quénéa et al., 2005a). According to Otto and Simpson (2006), this value resembles that obtained for grassland soils rather for pine forest ones for which values < 1 were found. This could indicate an important contribution of suberin from the undergrowth in addition to that of the cutin previously suggested.

4.3. Origin of aromatic compounds

Apart from the tentatively identified (**14**, **15**) and unidentified (**17**-**19**) aromatic compounds which were mainly found in the extract from microwave irradiation in 0.1 M HCl (Fig. 2b), similar aromatic compounds were present in all the extracts (Fig. 2; Table 1). These compounds were predominantly guaiacyl, *p*-hydroxy and syringyl phenols and were generally considered to be derived from lignin (eg. Brunow, 2001). However, substituted dihydroxyphenols (e.g. **9**, detected in significant amounts in the extract from microwave irradiation in H_2O and 0.1 M HCl, and **10**, present in the extract from microwave irradiation in 1 M KOH) could also originate from (condensed) tannins (e.g. Galletti et al., 1995; Nierop et al., 2005). *p*-Coumaric (**12**), ferulic (**16**) acids and 4-hydroxybenzaldehyde (**1**) can also arise from the aromatic domain of suberin (Walton, 1990; Bernards, 2002) to which they could be linked via ester bonds (Bernards and Lewis, 1998). As in the case of monosaccharides, the presence of these aromatic compounds in the extract from microwave irradiation in H_2O suggests that lignin monomers were present as such in the ROM. They could be adsorbed on (or entrapped in) the macromolecular structure of the ROM or they could form molecular associations with carbohydrates and could not be released upon extractions and hydrolyses of the isolation process. The relative abundances of guaiacyl and syringyl units increase upon microwave irradiation in 0.1 M HCl. The units released upon these conditions are likely present as arylglycerol-β-aryl ether structures, such structures being acid-hydrolysable (Adler et al., 1957; Lundquist and Lundgren, 1972). The tentative structures attributed to compounds **8**, **14**, **15, 17, 19** present in the extract from microwave irradiation in 0.1 M HCl (Fig. 3), could support this suggestion. Indeed similar types of ketones were found upon acid degradation of lignin (Adler et al., 1957; Lundquist and Lundgren, 1972). On the other hand, microwave irradiation in KOH leads to an increase in the relative abundances of *p*-coumaric, and ferulic acids. This suggests that these acids were predominantly in an ester-linked form in the ROM. They could be present esterified to lignin-polysaccharide complex or in the aromatic domain of suberin (e.g. Kolattukudy, 2001). The predominance of the guaiacyl over the syringyl units in all the extracts is consistent with the dominant gymnosperm vegetation. However, the significant amounts of *p*-hydroxyphenyl units, including high amount of *p*-coumaric acid, and ferulic acid, particularly in the extract from microwave irradiation in KOH, suggests a significant non-woody angiosperm undergrowth (likely gramineous plants) influence.

Interestingly is the presence of cyclodimers of *p*-coumaric acid (**20**) in the extract from microwave HCl and KOH hydrolyses. To our knowledge, these aromatic compounds have never been reported in soil lipids or in degradation products of soils or non-hydrolysable OM from soils. Cyclodimers of *p*-coumaric (or ferulic) acid have been reported as constituents of gramineous plant cell walls (Krauze-Baranowska, 2002 and references therein). They are thought to be synthesized by photodimerisation of *p*-coumaric acid and to reduce the biodegradability of the cell walls (Ford and Hartley, 1989). Their presence supports a contribution of gramineous-derived compounds to the ROM although cyclodimerisation of *p*-coumaric acid under microwave irradiation cannot be completely disregarded.

4.4. Pyrolysis of the residues. Comparison of microwave extractions/hydrolyses with pyrolysis

In good agreement with earlier pyrolytic studies of the same ROM sample (Quénéa et al., 2005a), the pyrochromatogram obtained by CuPy/GC-MS of the ROM (Fig. 4a) is dominated by products commonly observed for the pyrolysis of cellulose and related carbohydrates (Pouwels et al., 1989; Pastorova et al., 1994, Gauthier et al., 2003) i.e. levoglucosan, levoglucosenone and 2,3-dihydrobenzofuran. A series of *n*-alkane/*n*-alkene doublets is present in high relative abundance. Two main origins are generally considered for these doublets, i.e. non-hydrolysable macromolecules of higher plants such as cutan or suberan (Tegelaar et al., 1989; 1995; Augris et al., 1998; Nierop, 1998) or lipids incorporated through covalent bonds into the recalcitrant macromolecular structures (Almendros and Sanz, 1992; Almendros et al., 1996). The distribution pattern of this series appears to result from a combination of a smooth distribution centered between C_{24} and C_{27} and separate C_{20}, C_{22} and C_{24} doublets. This suggests that the *n*-alkane/*n*-alkene doublets likely derive from the two aforementioned origins, the dominant C_{20}, C_{22} and C_{24} doublets likely originating from incorporated lipids. The series of *n*-alkan-2-ones observed here, was not detected by Quénéa et al. (2005a) in the pyrolysate of the same ROM sample, while it was observed, with a similar distribution, in the pyrochromatogram of the double-shot pyrolysis of the same ROM sample (Quénéa et al., 2006b). The differences in the techniques (e.g. magnetic wire vs magnetic tubes) and/or temperature (610 °C in the present study, 650 °C for Curie point pyrolysis and 600 °C for the double-shot pyrolysis) could explain these discrepancies. The origin of *n*-alkan-2-ones is still a subject of speculation. β -Oxidation of *n*-alkanes (e.g. Cranwell et al., 1987; Jaffé et al., 1996), β-oxidation and subsequent decarboxylation of *n*-alkanoic acids (Amblès et al., 1989) and direct inputs of *n*-alkan-2-ones naturally occurring in

living organisms (e.g. Volkman et al., 1983; Quénéa et al. 2006b) have been postulated as possible origins for *n*-alkan-2-ones present in sediments, soils or recalcitrant organic matter. Moreover, as far as we are aware, such ketone doublets have not been previously reported in pyrolysates. Their presence would indicate that the keto group is not formed upon pyrolysis, the doublets being formed in a similar way as the *n*-alkane/*n*-alkene doublets.

Several phenolic compounds generally considered to originate from pyrolysis of lignin (e.g. Gauthier et al., 2003) were also detected in high relative abundances (Fig. 4a; Table 2). It should be noted that most of the phenolic compounds detected may be related to guaiacyl units. Only phenol (**3**, Table 2), which displays a rather low relative abundance, may be related to *p*-coumaric acid. However the composition of the extracts, especially that from microwave 1 M KOH hydrolysis (Fig 2c) indicates a substantial contribution of *p*-coumaric acid to the ROM. In addition, the methylated form of *p*-coumaric acid is present as one of the major aromatic compounds in the CuTHM pyrolysate of the ROM (Fig. 5; Table 3). This indicates that CuPy technique likely underestimates the contribution of *p*-coumaric acid to the ROM.

The strong decrease in the relative abundances of the compounds derived from polysaccharides (**1**, **2b**, **6**, **8**, **15;** Table 2) in residues from the microwave HCl hydrolyses (Figs. 4b, c) is consistent with the predominance of monosaccharides in the extracts from microwave HCl hydrolyses. In spite of drastic acid hydrolyses and extractions applied for the ROM isolation, mono- and oligosaccharides are still present in significant amounts in the ROM. The presence of such compounds in the ROM could be explained by the fact that mono-and oligosaccharides belong to molecular associations (e.g. lignin-carbohydrate complex) which could not be disrupted by the conventional heating used during the isolation process. However, these molecular associations containing highly polar carbohydrates could be disrupted upon microwave irradiation. Therefore it appears that carbohydrates could be released from covalent or physicochemical entrapment. However, although greatly reduced, the polysaccharidic contribution to the ROM was not completely removed, even after microwave irradiation in 1 M HCl (Fig. 4c). The microwave HCl hydrolyses do not result in a noticeable decrease in the relative abundance of compounds considered to derive from lignin (i.e. **2a**, **3**, **5**, **7**, **9**-**14**, **16**; Table 2). Similar trend is observed for the *n*-alkane/*n*-alkene and *n*-alkanon-2-one/*n*-alken-2-one doublets. This is consistent with the low relative abundances of aromatic and aliphatic compounds present in the extract from microwave irradiation in HCl (Fig. 2b).

The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolyses reveals a strong decrease in the relative abundances of *n*-alkane/*n*-alkene and *n*-alkan-2-one/*n*-alken-2-one doublets (Fig. 4d). Furthermore, among the series of *n*-alkane/*n*-alkene doublets, the relative abundances of C_{22} and C_{24} homologues (assumed to to derive from covalent linked lipids) tend to decrease and fall in the same range as the whole series. This could be correlated to the high relative abundances of C_{22} and C_{24} *n*-alkan-1-ols and/or ω-hydroxy alkanoic acids observed in the extract from microwave 1 M KOH hydrolysis (Fig 2c) and in the CuTHM pyrolysates of the ROM and the residues obtained after microwave HCl hydrolyses (Figs. 5a, b, c). This suggests that at least part of the predominant C_{22} and C_{24} *n*-alkane/*n*-alkene doublets originate from such C_{22} and C_{24} alcohols and/or ω-hydroxy alkanoic acids (or from the moieties (eg. esters) from which they derive).

The microwave irradiation in 1 M KOH results in a substantial decrease in the relative abundances of phenolic compounds with the exception of 2-methoxyphenol (**5**) and 4-methyl-2-methoxyphenol (**7**) which largely dominate the pyrochromatogram (Fig. 4d). These two compounds can be related to guaiacyl units of lignin

The distribution pattern of the compounds released upon CuTHM of the ROM (Fig. 5a) is in good agreement with that obtained by Quénéa et al. (2005a) for the same sample. The pyrochromatogram is dominated by aliphatic and aromatic compounds considered to derive respectively from cutin and/or suberin and lignin. However, notable differences between the two distributions are observed. *n*-Alkan-1-ols, mid-chain hydroxy alkanoic acids and cyclodimers of *p*-coumaric acid observed in the present CuTHM pyrolysate have not been identified by Quénéa et al. (2005a). Mid-chain hydroxy alkanoic acids (**20**-**26**; Table 3) were tentatively identified on the basis of their mass fragmentation and by selecting the carbon chain lengths found for this type of compounds in the extracts from the microwave irradiations (ie C_{15}, C_{16} and C_{18}). The proposed structures (Fig.6) involve the presence of non-methylated hydroxyl groups in some of these compounds. Providing that these structures are correct, this indicates that hydroxyl groups are only partially methylated upon CuTHM. The partial methylation of hydroxyl groups have been reported in earlier studies (de Leeuw and Baas, 1993, Kralert et al., 1995). It has been suggested that partial methylation of alkanols upon CuTHM is due to competition between methylation and thermovaporization of these compounds (de Leeuw and Baas, 1993, Kralert et al., 1995). The aromatic compounds are dominated by the methylated counterparts of vanillic (**14**) and *p*-coumaric (**17**) acids (Fig. 5a; Table 3).

Contrary to CuPy, typical products related to carbohydrates are not detected in the CuTHM pyrolysate of the ROM. This is consistent with previous observations (Quénéa et al., 2005a). It must also be noted that the major aromatic products from the CuPy (**5** and **7**; Fig. 4) appear in CuTHM pyrolysate as rather minor products (**4** and **6**; Fig 5) whereas vanillic (**14**) and *p*-coumaric (**17**) acids largely dominate. This highlights the much higher efficiency of CuTHM when compared to CuPy for the detection of lignin-derived compounds.

The CuTHM pyrolysates of the residues obtained after microwave HCl hydrolyses (Figs. 5b, c) reveal a decrease in the relative abundances of aliphatic compounds. By contrast, and as already noticed for the CuPy pyrolysates of these residues, no notable change in the relative abundances of aromatic compound is observed. The decrease in the relative abundances of ω-hydroxy acids and, to a lesser extent that of *n*-alkan-1-ols, appears to be more pronounced than that of *n*-alkanoic acids. For example, when the CuTHM pyrolysate of the ROM and that of the residue from microwave 1 M HCl hydrolysis are compared (Fig. 5a, c), the C_{22} ω-hydroxy acid to C_{22} *n*-alkanoic acid and C_{22} *n*-alkan-1-ol to C_{22} *n*-alkanoic acid ratios are respectively ca. 3 times and 2 times lower in the latter pyrolysate, while the C_{22} ω-hydroxy acid to C_{22} *n*-alkan-1-ol ratio is only 1.3 times lower. The same trend is observed for C_{24} homologues (Table 4). It appears that *n*-alkanoic acids are more difficult to release by microwave HCl hydrolyses. This is consistent with the distribution of the aliphatic compounds in the extract from microwave HCl hydrolyses. Indeed, in these extracts, the aliphatic compounds were dominated by ω-hydroxy acids and *n*-alkan-1-ols. This result suggests the existence of different pools for *n*-alkanoic acids on the one hand, and for ω-hydroxy acids and *n*-alkan-1-ols on the other hand.

In good agreement with the composition of the extract from microwave 1M KOH hydrolysis, the decrease in the relative abundances of aliphatic compounds is especially marked in the CuTHM pyrolysate of the final residue (Fig. 5d). However, in contrast to the CuTHM of the residues from microwave HCl hydrolyses, the decrease in the relative abundances of the different aliphatic compounds appears to be uniform (Table 4). Regarding the aromatic compound class (**1**-**18**; Figs 5a-d) no noticeable change in the relative abundance of its constituents were observed between the CuTHM pyrolysates of the ROM and the residues **2**, **3** and **5**. The final residue obtained after microwave extractions and hydrolyses appears to be predominantly composed of aromatic compounds with vanillic and *p*-coumaric acids as the major components. This indicates that at least part of lignin-derived constituents are resistant to microwave acid and base hydrolyses. By contrast, these constituents can be released by

thermal cracking. However, the microwave irradiation of commercial lignin (Lignin, hydrolytic, Aldrich) in 0.1 M NaOH resulted in almost 100% degradation (unpublished results). This suggests that lignin in the ROM is efficiently protected.

At this stage, work is preliminary and the mechanistic aspects of microwave hydrolyses of such recalcitrant macromolecular material must be studied and the range of samples expanded.

5. Conclusions

The recalcitrant organic matter (ROM) isolated from a forest soil was submitted to sequential microwave assisted extractions and/or hydrolyses in H_2O, HCl and KOH, resulting in ca. 35% degradation of the initial ROM. The overall extracts consisted in ca. 10% carbohydrates and ca. 25% aliphatic and aromatic compounds.

The differences between the distributions in the extracts from microwave irradiation in H_2O and in HCl suggest that acid hydrolysis of oligo(poly)saccharides and oligo(poly)peptides occurred upon microwave irradiation in HCl.

Products resulting from microwave irradiation in H_2O and in HCl are strongly dominated by glucose. The resistance of carbohydrates to the acid hydrolyses and extractions performed during the isolation of the ROM is thought to originate from the presence of carbohydrate-containing molecular associations. These molecular associations contain polar functions suitable for localized superheating effects under microwave irradiations and subsequent disruption, whereas they are resistant to conventional heating.

The distribution of compounds released from microwave irradiation in KOH indicates an important suberin- and cutin-derived contribution to the ROM. Moreover, this distribution, together with the value of the suberin/cutin ratio suggests a significant input of both cutin and suberin from the undergrowth.

Lignin-derived compounds, present in the all the extracts from microwave hydrolyses are dominated by guaiacyl moieties, in agreement with the predominance of pines at the study site. A significant contribution from the non-woody angiosperm undergrowth to the ROM is also suggested by the presence of *p*-hydroxyphenyl units (mainly *p*-coumaric acid) together with that of cyclodimers of *p*-coumaric acid. Two types of lignin monomers are shown to be engaged in different linkages (ether vs ester) as they are specifically released through microwave HCl or KOH hydrolyses.

The composition of the CuPy and CuTHM pyrolysates of the residues obtained after microwave irradiations were consistent with the distribution patterns of the corresponding extracts.

Finally, the present study illustrates how complementary the microwave irradiation and pyrolysis methods are. Each method presents its own advantages and drawbacks. Microwave assisted extractions and hydrolyses afford quantitative (e.g. yield of degradation) as well as qualitative data (e.g. nature of constituent monosaccharides) which cannot be obtained through on-line pyrolysis. In addition, this technique appears to provide a more accurate measure of the contribution of cutin-derived constituent (i.e. mid-chain hydroxy alkanoic acids) due to the incomplete methylation of the hydroxyl groups occurring during CuTHM pyrolysis. On the other hand, contrary to pyrolysis, microwave assisted hydrolyses appears to be unable to release the major part of the lignin-derived constituent of the ROM. On-line pyrolysis is much less time consuming. However, it is necessary to perform both CuPy and CuTHM to have a holistic view of the composition of the ROM (e.g. carbohydrate only detected in CuPy pyrolysate and polar constituents only detected in CuTHM pyrolysate). Microwave assisted extractions and/or hydrolyses appear as particularly suitable for the analysis of complex matrices and could be an attractive and complementary technique to pyrolysis methods.

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836

Table 1

Compounds identified in the extracts from the sequential microwave irradiation treatments (black numbers in Figure 2).

1	4-hydroxybenzaldehyde
2	3-hydroxybenzoic acid
3	3(4)-hydroxyacetophenone
4	4-hydroxybenzoic acid
5	4-hydroxy-3-methoxybenzaldehyde
6	4-hydroxy-3-methoxyacetophenone
7	4-hydroxy-3-methoxybenzoic acid (vanillic acid)
8	3-(4-hydroxy-3-methoxyphenyl)-propan-2,3-dione (see Fig. 3a)
9	3,4-dihydroxybenzoic acid
10	3,5-dihydroxybenzoic acid
11	4-hydroxy-2,6-dimethoxyacetophenone
12	3-(4-hydroxyphenyl)-2-propenoic acid (*p*-coumaric acid)
13	4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)
14	3-(4-hydroxy-3-methoxyphenyl)-3-oxopropanol (see Fig. 3b)
15	3-(4-hydroxy-3-methoxyphenyl)-3-oxo-1-propen-1(2)-ol (see Fig. 3c)
16	3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid)
17	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3d)
18	unidentified (see Fig. 3e)
19	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3f)
20	Cyclodimers of *p*-coumaric acid (see Fig. 3g)
21	9-hydroxy pentadecanoic acid
22	10-hydroxy pentadecanoic acid
23	8/9/10-hydroxy octadecanoic acid
24	6/7 hydroxy pentadecanedioic acid
25	9,15-dihydroxy pentadecanoic acid
26	7/8-hydroxy hexadecanedioic acid
27	8/9/10,16-dihydroxy hexadecanoic acid
28	9,10-dihydroxy octadecanoic acid
29	9/10/11,18-dihydroxy octadecanoic acid
30	9,10,18-trihydroxy octadecanoic acid

Table 2

Compounds identified in the 610 °C Curie point pyrolysates of the ROM and residues remaining after microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers in Figure 4)

1	2-furancarboxaldehyde
2a + 2b	benzaldehyde + methylfurancarboxaldehyde [a]
3	phenol
4	acetophenone
5	2-methoxyphenol
6	levoglucosenone
7	4-methyl-2-methoxyphenol
8	dihydrobenzofuran
9	4-ethenyl-2-methoxyphenol
10	3-methoxy-4-hydroxybenzaldehyde
11	4-(propen-2-yl)-2-methoxyphenol
12	3-methoxy-4-hydroxyacetophenone
13	3-methoxy-4-hydroxybenzoic acid methyl ester
14	3-methoxy-4-hydroxyphenylacetone
15	levoglucosan
16	3,4-dimethoxyphenylacetone [b]

[a] The ratio of **2b**/**2a** estimated through the intensity of peaks at *m*/*z* 110 and 106 decreases from ca. 1.8 for the ROM to ca. 0.9 and 0.2 for the residues from microwave hydrolyses in 0.1 M and 1 M HCl respectively. [b] tentatively identified (*m*/*z* 123, 151 (base peak), 194)

Table 3

Compounds identified in the 610 °C Curie point thermally assisted hydrolysis and methylation pyrolysates of the ROM and residues remaining after microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers in Figure 5)

1	methoxy toluene
2	2-methoxyphenol
3	benzoic acid
4	1,2-dimethoxybenzene
5	4-methoxy ethenylbenzene
6	3,4-dimethoxytoluene
7	4-methoxybenzaldehyde
8	1,2,3-trimethoxybenzene
9	3-methoxybenzoic acid
10	3,4-dimethoxy ethenylbenzene 1,2,4-trimethoxybenzene 4-methoxybenzoic acid
11	3,4,5-trimethoxy ethenylbenzene
12	3,4-dimethoxybenzaldehyde (vanillic aldehyde)
13	3,4-dimethoxyacetophenone (acetovanillone)
14	3,4-dimethoxybenzoic acid (vanillic acid)
15	3,4,5-trimethoxybenzaldehyde (syringaldehyde)
16	3,4-dimethoxybenzeneacetic acid
17	3-(4-methoxyphenyl)-2-propenoic acid (*p*-coumaric acid)
18	3,4,5-trimethoxybenzoic acid (syringic acid)
19	3-(3,4-dimethoxyphenyl)-2-propenoic acid (ferulic acid)
20	9/10,16-dimethoxy hexadecenoic acid (Fig. 6a)
21	8/9/10,16-dimethoxy hexadecanoic acid (Fig. 6b)
22	7/8/9-methoxy-15-hydroxy pentadecanoic acid (Fig. 6c)
23	7/8-dimethoxy hexadecanedioic acid (Fig. 6d)
24	8/9-methoxy-16-hydroxy hexadecanoic acid (Fig. 6e)
25	9,18-dimethoxy octadecanoic acid (Fig. 6f)
26	9/10-hydroxy-18-methoxy octadecanoic acid (Fig. 6g)
27	cyclodimers of *p*-coumaric acid

Table 4

ω-Hydroxy acid/*n*-alkanoic acid, ω-hydroxy acid/*n*-alkan-1-ol and *n*-alkan-1-ol l/*n*-alkanoic acid ratios calculated from the peak areas of GC-MS traces of CuTHM pyrolysates.

	ROM	Residue after microwave irradiation in 1 M HCl	Residue after microwave irradiation in 1 M KOH
C_{22} ω-hydroxy acid/C_{22} *n*-alkanoic acid	7.4	2.4	2.4
C_{22} ω-hydroxy acid/C_{22} *n*-alkan-1-ol	2.3	1.7	2.1
C_{22} *n*-alkan-1-ol l/ C_{22} *n*-alkanoic acid	3.2	1.4	1.1
C_{24} ω-hydroxy acid/C_{24} *n*-alkanoic acid	4.6	1.5	1.6
C_{24} ω-hydroxy acid/C_{24} *n*-alkan-1-ol	2.6	1.7	1.9
C_{24} *n*-alkan-1-ol l/ C_{24} *n*-alkanoic acid	1.8	0.9	0.8

879

880 Figure Captions

881

882 Fig. 1. Sequential microwave irradiation treatments of the ROM from the forest soil sample.

883

884 Fig. 2. Total ion current (TIC) traces of the extracts from microwave irradiation in (**a**) H_2O;
885 (**b**) 0.1 M HCl; (**c**) 1 M KOH. The compounds were identified as methyl ester and
886 trimethylsilyl ether derivatives. Black numbers correspond to compounds listed in Table 1.
887 White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-1-ols; ●:
888 ω-hydroxy acids; ▽: *n*-alkanedioic acids; **S**: mono- or disaccharides; ✷: pollutant. When two
889 different peaks are labelled with the same number (e.g. **7** or **9**), that with the highest retention
890 time corresponds to the trimethylsilyl ester and ether derivative.

891

892 Fig. 3. Mass spectra of tentatively identified and unidentified aromatic compounds found in
893 the organic extracts from the microwave extractions and hydrolyses (Table 1 for peak
894 annotations). The analysis of compounds as trimethylsilyl ester and ether derivatives reveals
895 (i) two compounds with the same retention times and mass spectra as compounds **8** and **15**,
896 indicating the absence of carboxyl groups in these compounds. (ii) a compound, at longer
897 retention time than compound **14**, with a mass spectrum exhibiting peaks at m/z 193, 223
898 (base peak), 325, 340 and 383. This suggests the presence of one carboxyl group for
899 compound **14**. Compound **17** has the molecular weight of a trimethylsilyl ether, trimethylsilyl
900 ester of methoxyhydroxybenzoic acid or trimethylsilyl ether, methyl ester of
901 dihydroxybenzoic acid. However the mass spectra of these latter compounds do not match
902 with that of compound **17**. Compounds **18** and **19** have the molecular weight of a
903 trimethylsilyl ether, trimethylsilyl ester of dihydroxybenzoic acid, but no match was found
904 between the mass spectra of these different compounds.

905

906

907 Fig. 4. TIC traces of 610 °C Curie point (CuPy) pyrolysate of (**a**) ROM and residues
908 remaining after microwave irradiation in (**b**) 0.1 M HCl; (**c**) 1 M HCl; (**d**) 1 M KOH. Black
909 numbers correspond to compounds listed in Table 2. White numbers correspond to carbon
910 chain length. ▼: *n*-alkanoic acids; ◇: *n*-alkane/*n*-alkene doublets; ◆: *n*-alkan-2-one/*n*-alken-
911 2-one doublets.

912

Fig. 5. TIC traces of 610 °C Curie point thermally assisted hydrolysis and methylation (CuTHM) pyrolysate of (**a**) ROM and residues remaining after microwave irradiation in (**b**) 0.1 M HCl; (**c**) 1 M HCl; (**d**) 1 M KOH. Black numbers correspond to compounds listed in Table 3. White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-1-ols; ●: ω-hydroxy acids; ▽: *n*-alkanedioic acids; ◇: *n*-alkane/*n*-alkene doublets; ✳: hexahydro-1,3,5-trimethyl-1,3,5-triazine, a by product from TMAH.

Fig. 6. Mass spectra of the tentatively identified mid-chain substituted ω-hydroxy alkanoic and alkanedioic acids found in the CuTHM pyrolysates of the ROM and residues from microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH. (Table 3 for peak annotation).

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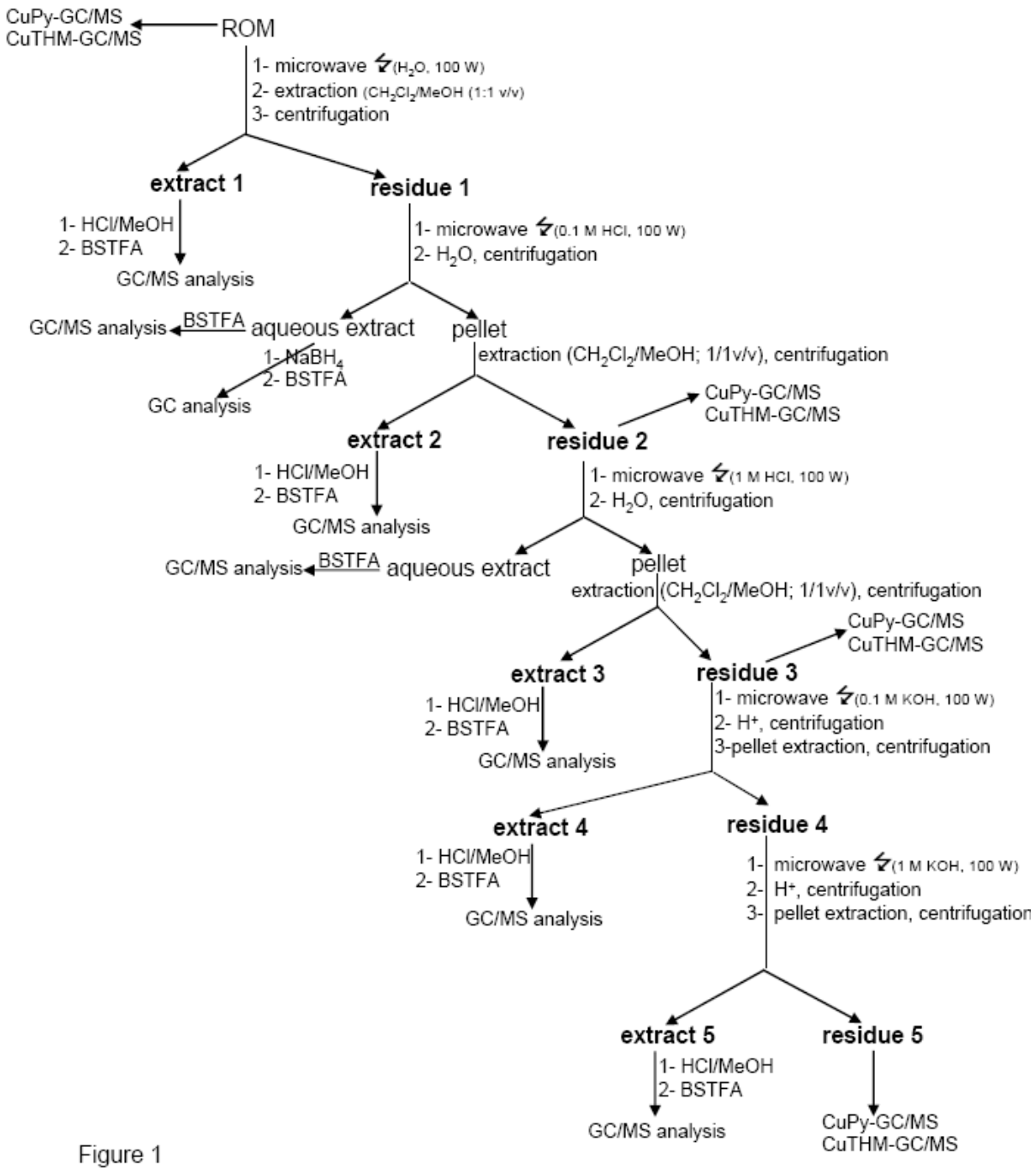

Figure 1

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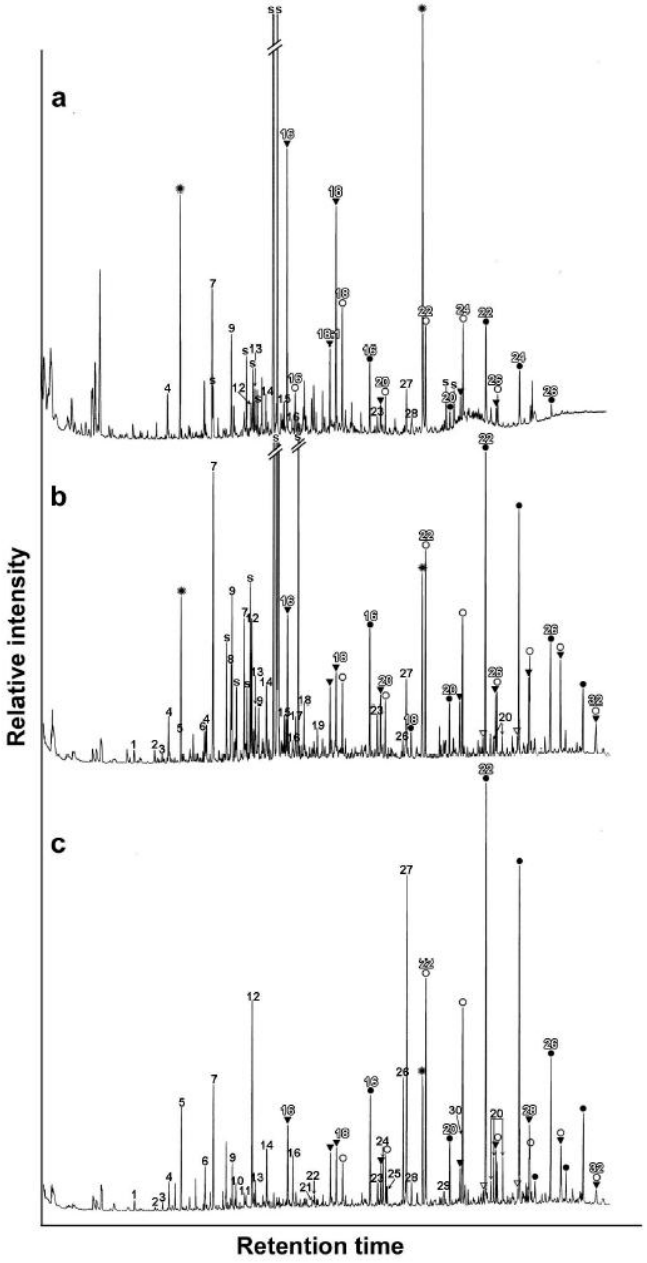

Figure 2

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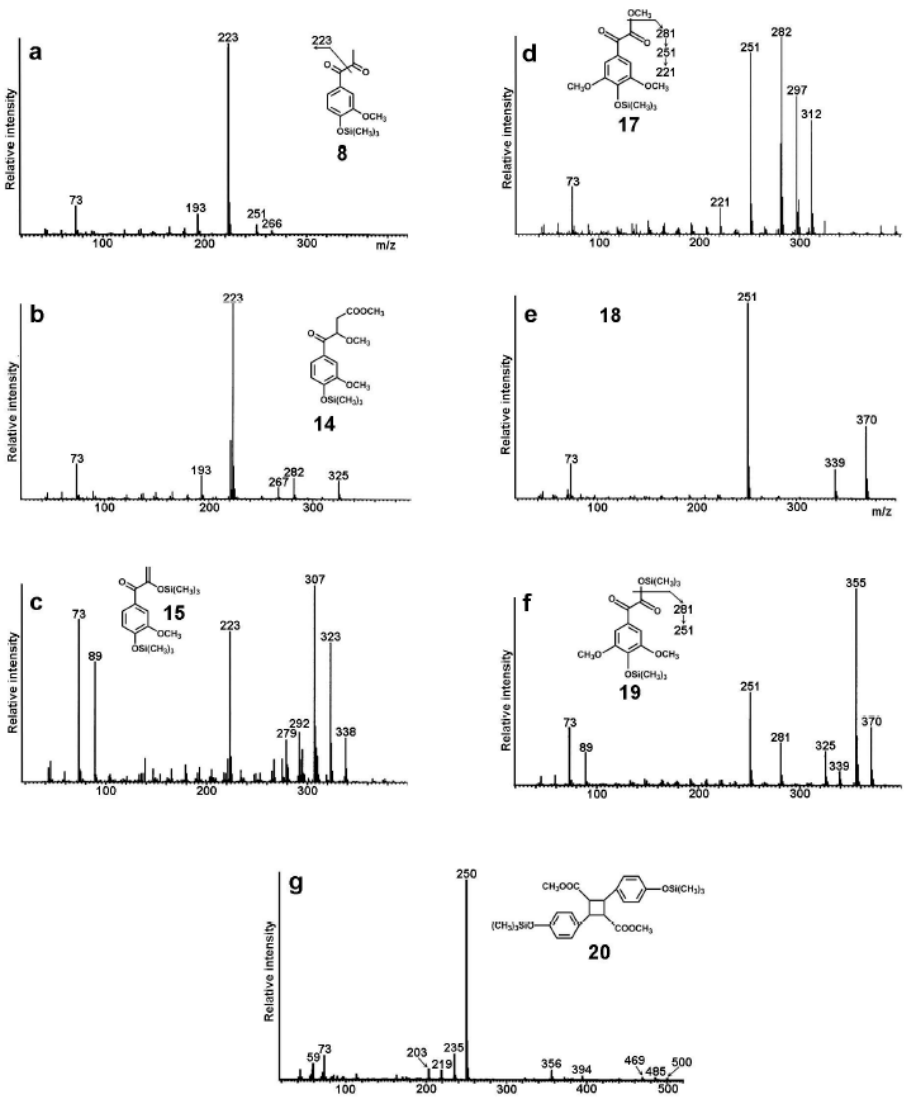

Figure 3

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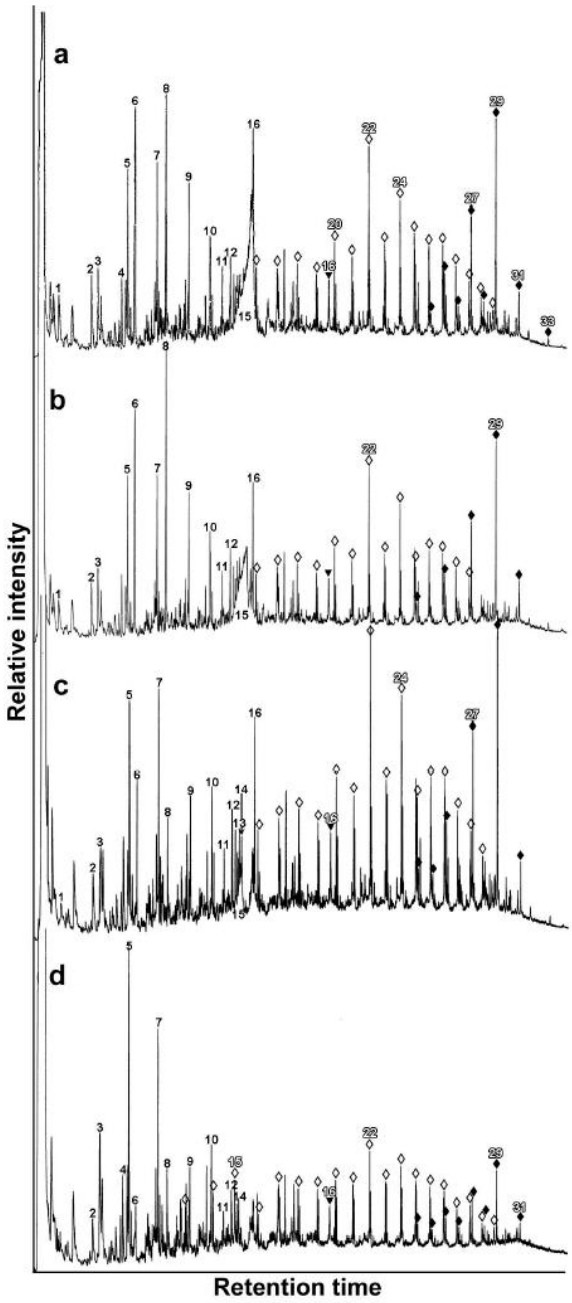

Figure 4

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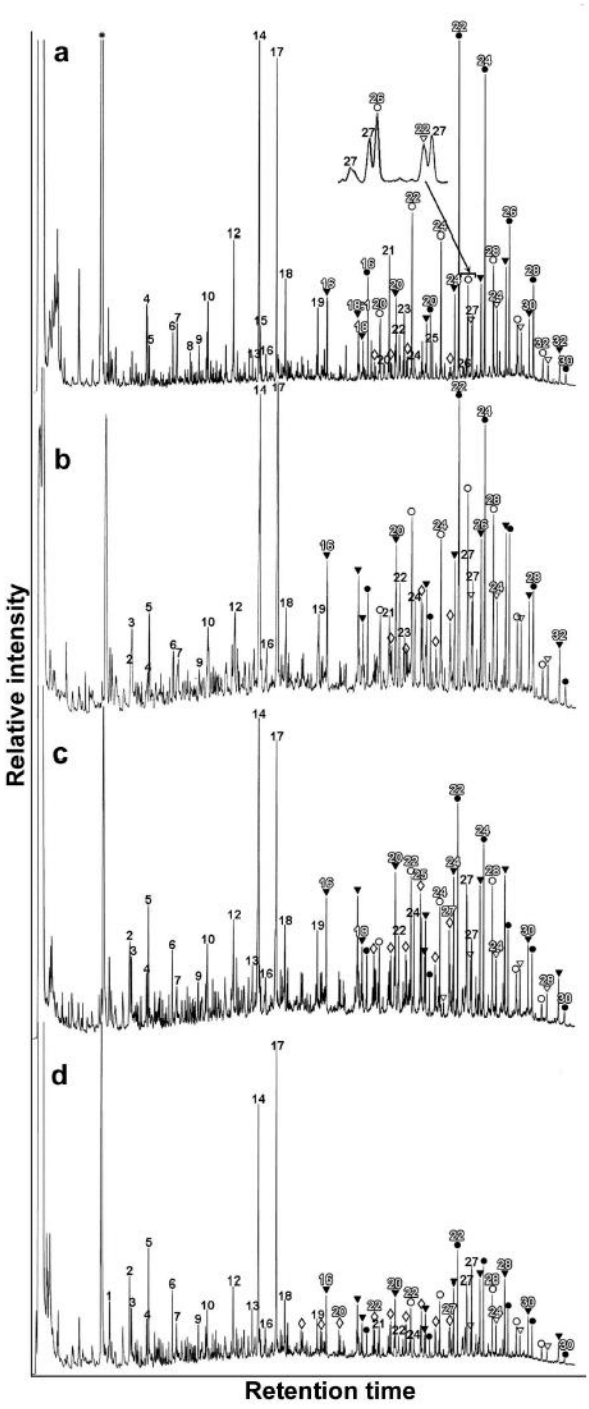

Figure 5

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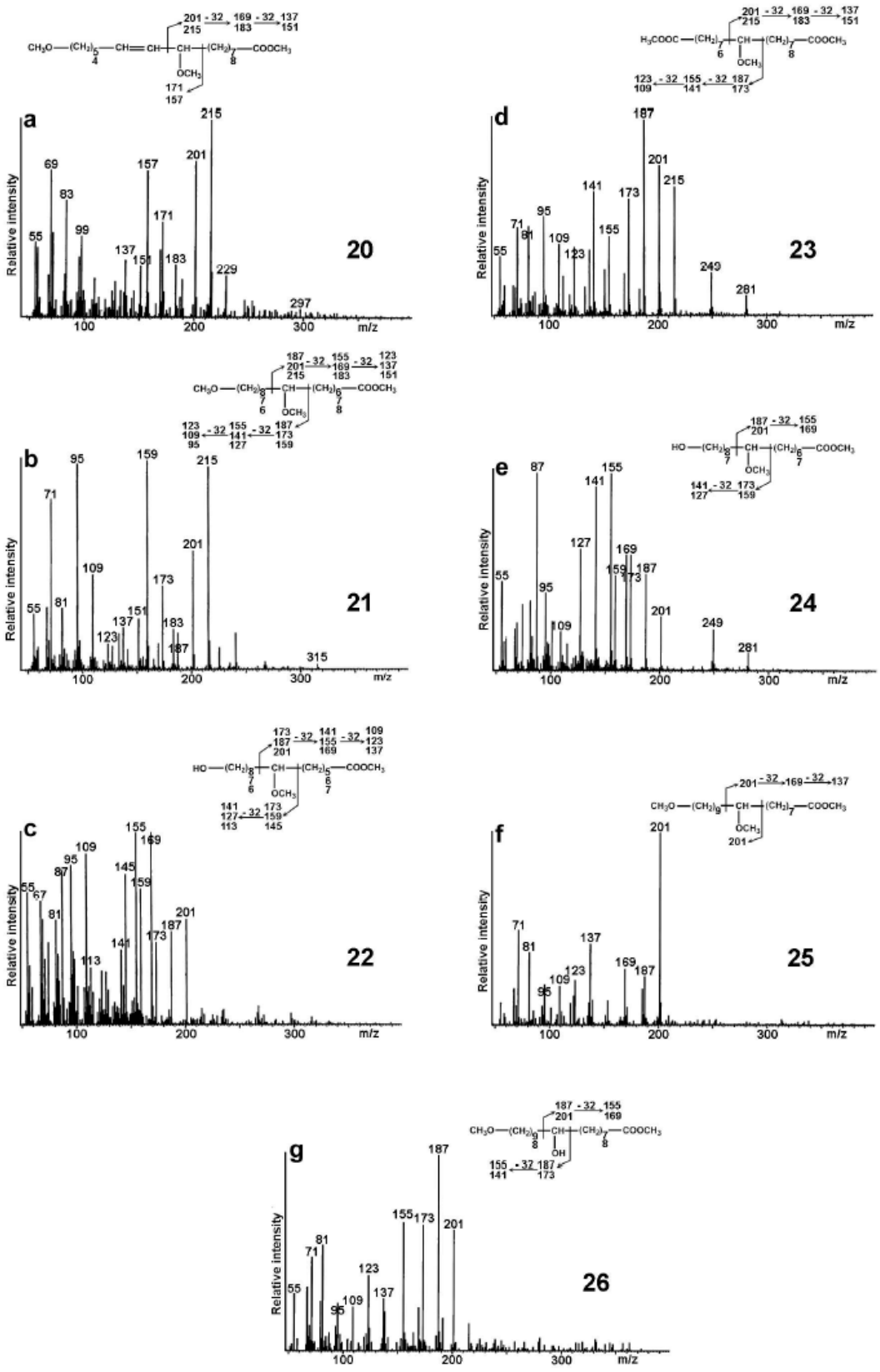

Figure 6

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